



## Immunomodulation by dietary $\beta$ -glucans in fish

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## Summary

$\beta$ -Glucans form a heterogeneous group of polysaccharides found as structural or storage components of bacteria, fungi, algae, lichens and plants. These polysaccharides are not present in animals, in which they are identified by receptors of the innate immune system and may potentiate immune responses. The present thesis provides a literature review of previous research on  $\beta$ -glucan and disease resistance/immune responses in fish. This is followed by a series of experiments elucidating the immunostimulatory potential and effects of a linear  $\beta$ -1,3-glucan (paramylon) from the alga *Euglena gracilis* orally delivered to rainbow trout exposed to bacterial and parasitic infection. The pathogens included in these studies were the Gram-negative bacterium *Yersinia ruckeri* and the protozoan parasite *Ichthyophthirius multifiliis*, being the causative agents of enteric redmouth (ERM) disease and white spot disease, respectively. Both diseases are responsible for considerable fish mortality and economic losses in aquaculture production.

Paramylon induced an increase in *I. multifiliis* resistance in rainbow trout, reducing the intensity of infection by approx. 25%. This beneficial effect was both time and dosage dependent and associated with an unusually high inclusion level of paramylon (5%) in feed. Improved resistance to *Y. ruckeri* infection was not achieved. Nevertheless, the expression, in head kidney of ERM vaccinated fish, of immune relevant genes, *i.e.* IL-1 $\beta$ , SAA, hepcidin, precerebellin, and lysozyme, was significantly modulated by continuous oral administration of paramylon. A significant interaction between paramylon and vaccination was found with regard to expression of TNF- $\alpha$ , IFN- $\gamma$  and the above mentioned genes. The significant differences in gene expression were mainly observed as down-regulations in vaccinated fish receiving paramylon compared to up-regulations or no regulation in vaccinated controls. The nature and importance of these paramylon induced differences in gene expression could not be settled, since they were not reflected in the survival curves, which were unaffected by oral administration of paramylon. A tendency towards a priming effect of paramylon on the plasma lysozyme activity response to infection, combined with a trend in the opposite direction towards a suppressing effect of paramylon on antibody response, may have masked any effect of the significant modulation of gene expression found.

Besides the observed effects related to paramylon, plasma lysozyme activity in response to *Y. ruckeri* challenge was observed as two distinct and equally potent peaks of increase. The first peak occurred shortly after exposure to *Y. ruckeri* (at day 3 post-challenge (p.c.)) and was shared by both vaccinated and unvaccinated fish indicating that plasma lysozyme activity is an important part of the first line of defence irrespective of vaccination status. The second peak, observed approximately two weeks after fish mortality had leveled off (at day 28 p.c.), was only observed in unvaccinated fish and suggested this innate immune mechanism to be important for controlling infection in fish not immunised by vaccination.

It was concluded that paramylon as applied in the present experimental work does not constitute an effective, oral immunostimulant during *Y. ruckeri* infections, but some protection against white spot disease is conferred. Further, clear effects detected on various immune parameters in rainbow trout call for further research on  $\beta$ -glucan induced effects on the intricate immune regulation and signalling in fish.